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Comparative study of the variability of chemical composition and antibacterial activity between two Moroccan endemic species essential oils: Origanum grosiiPau & Font Quer and Origanum elongatum (Bonnet) Emberger & Maire

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Abstract

Nowadays, plants have become one of the promising sources of chemical compounds that are used in several applications especially in the medicinal. This study aims to illustrate the variability of the chemical composition and the antibacterial activity essential oils of two endemic species in Morocco: Origanum grosii Pau & Font Quer and Origanum elongatum (Bonnet) Emberger & Maire. Each species was collected from two different altitudes in the same region "Taounate" (Morocco). The chemical composition as well as the antibacterial activity of essential oils extracted from dried plants via hydrodistillation was evaluated. Gas chromatography-Mass spectrometry (GC-MS) results showed that the essential oils contain forty-three components, representing around 99% of the total oil for all the tested samples. Carvacrol and Thymol are major compounds for both species. Staphylococcus aureus had a higher sensitivity for the first altitude in both species (MIC=MBC=1.3mg/mL), while Pseudomonas aeruginosa and Acinetobacter baumannii were the higher sensitive for the second altitude with (MIC=MBC=2.5mg/mL). In the light of these findings, Knowledge of the chemical composition and antibacterial activity of essential oils with environmental factors (altitude, edaphic, climatic and genetic factors…) is a very important quality criterion for their marketing and contributes to their valorization as a source of antibiotics after testing its toxicity on humans.

Keywords: Origanum grosii, Origanum elongatum, antibacterial activity, chemical composition, Staphyloccocus aureus.

1. Introduction

Bacteria is among the main causes of several diseases, the fight against these pathologies has become an urgent subject for scientific researchers, especially for certain bacteria that become resistant to antibiotics. This resistance, as well as the appearance of side effects for some antibiotics, has led a search for new antimicrobial agents from plants because of their advantages such as low toxicity, natural abundance and pharmacological activities [1].

According to the previous researchers, essential oils showed potentialities as beneficial plant-extracts in many applications, including folk medicine, food flavoring, fragrance, and pharmaceutical industries [2,3]. The food industry uses synthetic preservatives to prevent the growth of foodborne, spoiling microbes, and also to extend the life of foods. However, the food industry is motivated to exploit essential oils as natural antimicrobial compounds [4]. Another study reported that the essential oils are also used in perfume and cosmetic industries for preservation and scenting for example perfumes, make-up products, dentistry, etc.

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[5,6]. Similarly, Paolini [7] illustrate, the potential of essential oils in biological agriculture against mushrooms and insects and for the fertilization of the soil.

Morocco is one of the richest botanical countries in the Mediterranean Basin with a high percentage (22%) of endemic species [8]. Several genera that contain many medicinal plants are included in the *Lamiaceae* family; the Oregano is one of this genus. Traditionally, a decoction of leaves and stems as well as their essential oils are widely used as a sedative, diuretic, sweater and antiseptic, also in the treatment of gastrointestinal diseases, menstrual problems, urinary tract disorders, respiratory disorders, and rheumatoid arthritis [8]. Generally, *Origanum* species are known for their ability to inhibit the growth of pathogenic bacteria which was reported in numerous studies [9,10]. Several works showed that *Origanum* essential oil possesses potential antibacterial activity with low minimum inhibitory concentration and minimum bactericidal concentration [10]. Many studies found that the pharmacological activities strongly depend on the chemical composition of the essential oil and its proportion, including Carvacrol, Thymol, 1,8-Cineole or other several constituents that containing a certain antimicrobial activity [11].

Origanum species are distributed throughout the Mediterranean area in Morocco as a wild plant that is grown on stony slopes at a wide range of altitudes [12]. It is represented by five species, three of them are endemic: *Origanum compactum*,*Origanum elongatum*, and *Origanum grosii* [13, 14]. The reported data by Jeannot [15] on *Origanum compactum* showed that hydrosol could have the traditional function to treat gastro-intestinal disorders and skin due to its low toxicity/irritation level. However, Chahbi [16] declared that this species had a very strong antibacterial activity. Concerning the *O.elongatum*, which was widely used for its therapeutic properties as a natural remedy for pain and abdominal spasms in North-Central Morocco [17]. While in the Middle Atlas (Jbel Bou Iblane), it is used to treat hepatic diseases [18], El Harsal [8] reported that this species had an important potential antibacterial. To the best of our knowledge, there are no practical works that evaluates the antibacterial activity of the species *O.grosii*.

On the other hand, several works have shown that harvested time [19], environmental factors such as the altitude, climatic and edaphic factors impact on the chemical composition of plants [20,21], in this context, the goal of this work was to study the variability and the difference of the chemical composition as well as antibacterial activity of Origanum *grosii* and *Origanum elongatum* harvested from different altitudes with some different environmental factors (temperature, soil,…) in the same region ”Taounate”. Another aim of this work is to make a comparative study of the chemical composition and antibacterial activity between the two endemic species.

2. Experimental

2.1. Plant material, extraction of essential oil

Collection of samples was done on different altitudes in the Taounate region, Morocco: *O.grosii:* **altitude1**:Taher souk (1331m), **altitude2**: Rhafssai (797m), and *O.elongatum* **Altitude1**: Aychtoum (923m), Altitude2**:** Bni Ahmed (1261m). These samples were collected during the period of the full flowering of plants (Jun-July 2016). *O.grosii* and *O.elongatum* were identified at the scientific institute of Rabat and the specimen voucher (99125, 99124 respectively) was deposited at the herbarium of the institute.

100 g of dried plant material was distilled with water (3h) using a Clevenger type apparatus [22].

2.2. *Essential* oil analysis

The essential oils were analyzed by GC using a flame ionization detector (FID), equipped with an HP-5 capillary column (30 m × 0.25 mm ID, 0.52 mm film thickness). The column temperature was programmed at 50 °C for 1 min then increased to 280°Cat 5 °C/min where it was held at isothermal for 1 min. The injector and detector temperatures were 250 °C and 280 °C, respectively using the nitrogen as the carrier gas at a flow rate of 1.2 ml/min. The injection volume was 0.1 ml of 1% solution in n-hexane.

GC-EI-MS analyses were performed with a CP-3800 gas chromatograph (Varian Inc., Palo Alto, CA) equipped with an HP-5 capillary column (30 m × 0.25 mm; film thickness 0.25 µm) and a Varian Saturn (Varian Inc., Palo Alto, CA) 2000 ion-trap mass detector. The oven temperature was programmed from 60 to 240°C at 3°C/min. The injector and transfer line temperatures were 220 and 240 °C respectively using the helium as carrier gas at a flow rate of 1 ml/min. The injection volume was 0.2 µl of a 1% hexane

solution, with a split ratio of 1:30. The acquisition parameters were in this way: full scan; scan time: 1.0 sec; scan range: 35-300 m/z; threshold: 1 count. The identification of the constituents was performed by the comparison of the retention times with those of pure authentic samples, comparing their LRI relative to the series of n-hydrocarbons, and on a computer matching them against commercial and home-made libraries of mass spectra, built from pure substances and components of known oils, and the MS literature data [23,24, 25, 26, 27, 28,].

2.3. Antibacterial activity

2.3.1. Microbial strains

The antibacterial activity of *O.elongatum* and *O.grosii* essential oils was evaluated against two bacteria Gram-positive isolates *Enterococcus faecalis* (P647), *Staphylococcus aureus*(P631 ; H2463) and six Gram negative: *Acinetobacter baumanii* (ASP195; HC2243; P582; HC2469; P658; HC2444), *Pseudomonas aeruginosa* (P612;HC2n418), *Enterobacter cloacae* (Bu5124), *Escherichia coli* (HC2420; P596), *Klebsiella pneumonia* (P596; Bu 4843) and *Serratia marcescens* (H2451;ASP222) (table1). The tested strains were obtained from the microbiology laboratory in Mohammed VI University hospital Centre in Marrakech, Morocco. The bacterial colonies were isolated in Petri dishes and incubated at 37 °C for 24h. The identification was performed using an automated system: The Phoenix BD system (BD Diagnostic Systems, Sparks, USA). *Escherichia coli* ATCC 25922 (American Type Culture Collection Center, Manassas, VA, USA) was used as quality control.

2.3.2. Disc diffusion method

The antibacterial activity of the tested essential oils was determined by the disk diffusion method. Briefly, 3 mL of a bacterial suspension (10^8CFU/mL of bacterial cells) were distributed on Petri dishes containing respectively Mueller-Hinton agar. Sterile paper disks (6 mm in diameter) were impregnated with 10µL of samples and placed on the inoculated agar. The disks without samples were used as a negative control. Reference drugs (25 antibiotics) were used as a positive control. The dishes were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the growth inhibition zone in centimeters [29].

Table 1: The studied bacteria and their sources

Bacteria	Gram Strains	Code	Isolation site
A.baumannii	Gram-	ASP 195	Bronchial aspiration
		HC2243	Blood culture
		P582	PUS (CHP)
		HC2469	Blood culture
		P658	PUS
		HC 2444	Blood culture
P.aeruginosa		P612	PUS
		HC2418	Blood culture
E.cloacae		Bu 5124	URINE
E.coli		HC2420	Blood culture
		P561	Pus
k.pneumoniae		P596	PUS
		Bu 4843	URINE
S.marcescens		H2451	Blood culture
		ASP222	Bronchial aspiration
E.faecalis	Gram+	P647	Pus
S.eureus		P631	Pus
		H2463	Blood culture

2.3.3. Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC and MBC)

The MIC is defined as the lowest concentration of essential oil that can inhibit microbial growth production by 90%. Antibacterial tests were performed according to the method reported by Pereira [30]. The essential oils were diluted with DMSO, noting that it was inactive against bacteria. The dilutions used in this work were: 10mg/mL (1,25%), 5mg/mL (0,63%), 2.5mg/mL (0,31%), 1.3mg/mL (0.16%), 0,65mg/mL (0.08%) and 0,33mg/mL (0.04%). 100µL of each dilution was then added to the test tubes containing 850µL of BHI and 50µL of the bacterial suspension. positive controls (bacterial suspension with DMSO and BHI) and negative control (physiological water with DMSO and BHI) were also prepared. The tubes were well stirred before being incubated at 37°C for 24 hours. The minimum inhibitory concentration (MIC) is considered for the first tube (lower concentration) that contains no bacterial development.

MBC is the lowest concentration of agents able to kill at least 99.99% of bacteria. To determine it, we incubated all the concentrations which are lower or equal to MIC in Petri dishes at 37°C for 24 hours [29]. MBC is also considered for the first Petri dish (lower concentration) that contains no bacterial development.

3. Results and Discussion

3.1. Essential oils composition

The chemical composition of *O.grosii* from both altitudes (OG1, OG2) and *O.elongatum* (OE1, OE2) are presented in table 2.

The chemical analysis revealed that forty-three components, which represents 99.74% and 99.84% for OG1(altitude1) and OG2 (altitude2) of the total *O.grosii*, respectively (Table2). The essential oils were highly dominated for OG1 and OG2 by Oxygenated monoterpenes (84.46%±4.22 and 76.50%±2.2 respectively). Therefore, the concentration of Oxygenated sesquiterpenes and Sesquiterpene hydrocarbons had a higher content for OG2 comparing to OG1.The lower content was presented for the Monoterpene hydrocarbons for OG2 which was more important as compared to OG1.

Overall, the main constituents of our essential oils for OG1 was Carvacrol (51.18%±1.30) which present an important value comparing to OG2 (43.20%±2.75). Secondly, the Thymol was higher in OG2 (24.24%±2.3) comparing to OG1 (18.62%±1.3). However, another element had less content for both altitudes as Linalol, Carvacrol methyl ether and β-Bisabolene.

The comparative analysis of our results with essential oil constituents which were reported in other studies done on the same species collected from different regions, the NW of Morocco [31] and Talassemtane park [32], declared that Carvacrol 42.96%- 47.7% respectively and thymol 15.45%-12.5%, respectively was also the major compounds, and had lower property than our species collected from the region Taounate. However, Bellakhdar and Idrissi [33] reported that *O.grosii* collected from Jbel Magou-NW of Morocco (1600m), was characterized by Thymol (35.5%), p-Cymene (28.5%), and γ-Terpinene (13.7%).This variation in the percentage of plant components was explained by several works, la Pergola [34] declared that the chemical composition can vary according to the harvesting period, to the geographical region, the variety, the age of the plant, the method of drying and the mode of extraction, other works reported that altitudes [19, 20], climatic differences, geographic conditions or all the other environmental factors [21] impact on the yield and the chemical composition, which demonstrate and explain this difference.

Concerning the *O.elongatum*, a total of forty-three components in OE1 (altitude1) and OE2 (altitude2) accounting to 99.62% and 99.77% respectively of its essential oils composition were detected (table2). As the results of *O.grosii*, the high percentage, for both altitudes, was represented by Oxygenated monoterpenes (80.73%±3.34 and 81.97%±4.03) followed by Monoterpenes hydrocarbons which were higher in OE2 comparing to OE1. Inversely to our results in *O.grosii*, the Sesquiterpene hydrocarbons and Oxygenated sesquiterpenes present the lower contents for both altitudes.

The main constituents of essential oils also in this species were Carvacrol (53.43%±3.54) in OE2, higher than OE1(36.48%±2.01). Secondly, Thymol in OE1 (29.09%±4) higher than OE2 (16.65%±2.34). Differently to *O.grosii*, the feeble contents were detected on ɣ-Terpinene , α-Terpineol and Caryophyllene oxide.

Comparing this values to previous work, done on *O.elongatum* essential oil harvested from the Hoceima region, Morroco showed that Thymol (63.44%), was the major compounds [6] While H.Oualili [35], following our results reported that the major compounds were Carvacrol (60.42%), and the same for Ramzi [36] who declared that the *O.elongatum* essential oil showed an important proportion of Carvacrol (67.34%).

A comparison of our results on the constituents between *O.grosii* and *O.elongatum* essential oils presented that Carvacrol had a higher proportion (53.43%±3.54) for the second altitude (OE2:1261m) in *O.elongatum* then *O.grosii* (51.18%±1.30) for the first altitude (OG1: 1331m), contrary to the Thymol was higher in *O.elongatum* (29.09%±4) in the first altitude (OE1: 923m) then *O.grosii* (24.24%±2.3) for the second altitude (OG2: 797m), which allows us to assume that the altitudes we worked on, gave us different results which were different to the other works from other regions, plainly because the difference in altitudes, regions and the environmental factors in general, impact on the concentration of the components, and this is confirmed in several works done on the other species of the same genus: *Origanum vulgare* which explained that the difference in percentages of the constituents essential oils is due to the environmental factors like altitude of plant growing habitat [37], developmental phase of the plant [38] and post-harvest management [39].

From these works, it can be seen that the difference of altitudes with climatic, edaphic, and genetic factors in the same region are among the main key reasons of chemical variations between our samples.

Table 2: Chemical composition of *O.grosii* and *O.elongatum* essential oils for both altitudes.

			Origanum grosii		*Origanum elongatum*	
			Tahar souk (1331m)	Ghafssai (797m)	Aychtoum (923m)	Bni Ahmed (1261m)
Compounds	Ir /apol	Ir /pol	Altitude1 (OG1)	Altitude2 (OG2)	Altitude1 (OE1)	Altitude2 (OE2)
α-Thujene	925	1031	0.1 ± 0.05	0.28 ± 0.1	0.48 ± 0.1	0.37 ± 0.2
α-Pinene	933	1031	0.1 ± 0.03	0.09 ± 0.02	0.19 ± 0.05	0.28 ± 0.1
Camphene	946	1075	0.19 ± 0.05	0.09 ± 0.05	0.58 ± 0.21	0.09 ± 0.05
Oct-1-en-3-ol	963	1448	0.29 ± 0.03	0.28 ± 0.1	0.87 ± 0.3	0.93 ± 0.22
Octan-3-one	966	1254	0.1 ± 0.08	0.1 ± 0.04	1.74 ± 0.4	0.93 ± 0.4
Octan-3-ol	981	1391	0.1 ± 0.06	-	0.39 ± 0.1	0.37 ± 0.1
Myrcene	984	1165	0.19 ± 0.1	0.65 ± 0.2	0.28 ± 0.06	0.28 ± 0.06
α-Phellandrene	1000	1170	-	0.19 ± 0.07	0.77 ± 0.2	0.09 ± 0.06
3-Carene	1007	1154	0.29 ± 0.1	0.46 ± 0.08	0.1 ± 0.03	0.47 ± 0.04
α-Terpinene	1012	1184	0.1 ± 0.05	0.19 ± 0.1	0.68 ± 0.1	0.75 ± 0.12
p-Cymene	1015	1275	1.15 ± 0.3	0.93 ± 0.2	0.29 ± 2.12	0.47 ± 1.23
Limonene	1023	1204	0.1 ± 0.02	0.37 ± 0.1	0.19 ± 0.06	0.28 ± 0.1
1,8-Cineole	1024	1214	0.81 ± 0.21	0.14 ± 0.05	0.58 ± 0.16	0.47 ± 0.21
γ-Terpinene	1052	1250	1.05 ± 0.3	0.09 ± 0.02	6.49 ± 1.06	8.85 ± 2.15
E-Hydrate sabinene	1055	1462	0.19 ± 0.06	0.28 ± 0.15	0.29 ± 0.06	0.28 ± 0.03
Nonen-3-ol	1065	1522	0.19 ± 0.1	0.09 ± 0.02	-	0.09 ± 0.02
E-2-Octen-1-ol	1063	1599	0.29 ± 0.1	1.02 ± 0.3	1.16 ± 0.26	0.09 ± 0.02
Fenchone	1072	1400	1.38 ± 0.61	1.3 ± 0.60	0.1 ± 0.02	0.19 ± 0.01
Z Linalol oxyde	1074	1441	0.1 ± 0.03	0.09 ± 0.08	-	0.09 ± 0.03
p-Cymenene	1074	1443	0.29 ± 0.1	0.37 ± 0.08	0.58 ± 0.02	0.09 ± 0.07
Terpinolene	1082	1286	1.15 ± 0.3	4.16 ± 0.1	0.1 ± 0.02	0.09 ± 0.01
Linalol	1086	1547	2.82 ± 0.74	1.80 ± 0.5	1.26 ± 0.6	0.98 ± 0.41
Camphor	1126	1514	1.53 ± 0.7	1.67 ± 0.8	1.74 ± 0.36	1.4 ± 0.41
Borneol	1152	1700	0.33 ± 0.1	0.56 ± 0.23	0.39 ± 0.16	0.28 ± 0.22
p-Cymen-8-ol	1164	1844	3.34 ± 0.92	0.65 ± 0.23	0.29 ± 0.03	0.47 ± 0.1
Terpinen-4-ol	1164	1600	1.00 ± 0.1	0.65 ± 0.12	1.84 ± 0.16	0.84 ± 0.22
α-Terpineol	1174	1694	0.67 ± 0.05	0.09 ± 0.05	3.92 ± 1.23	3.82 ± 1.06
Thymoquinone	1216	1571	0.29 ± 0.05	0.19 ± 0.08	0.19 ± 0.03	0.37 ± 0.01
Carvacrol methyl ether	1228	1599	2.01 ± 0.5	3.79 ± 0.55	0.97 ± 0.04	0.93 ± 0.02
Thymol	1278	2180	**18.62 ± 1.3**	**24.24 ± 2.3**	**29.09 ± 4**	**16.65 ± 2.34**
Carvacrol	1288	2207	**51.18 ± 1.30**	**43.20 ± 2.75**	**36.48 ± .01**	**53.43 ± 3.54**
Eugenol	1334	2161	0.76 ± 0.2	0.09 ± 0.05	0.1 ± 0.04	0.09 ± 0.01
Trans Caryophyllene	1420	1603	0.19 ± 0.03	2.31 ± 1.2	1.16 ± 0.06	1.12 ± 0.05
Aromadendrene	1439	1608	1.15 ± 0.2	0.09 ± 0.02	0.19 ± 0.05	0.37 ± 0.11
α-Humulene	1452	1660	0.1 ± 0.03	0.19 ± 0.04	0.1 ± 0.02	0.09 ± 0.04
Bicyclogermacrene	1493	1732	0.29 ± 0.15	0.93 ± 0.1	0.19 ± 0.1	0.09 ± 0.07
β-Bisabolene	1503	1731	2.67 ± 0.91	3.24 ± 0.69	0.1 ± 0.06	0.09 ± 0.05
γ-Cadinene	1508	1756	0.1 ± 0.02	0.28 ± 0.1	0.1 ± 0.02	0.28 ± 0.05
Thymohydroquinone	1516	2176	0.48 ± 0.13	0.09 ± 0.03	0.29 ± 0.14	0.19 ± 0.14
δ-Cadinene	1517	1756	0.67 ± 0.1	1.02 ± 0.1	0.29 ± 0.05	0.09 ± 0.1
Spathulenol	1565	2120	1.72 ± 0.4	1.11 ± 0.13	0.1 ± 0.03	0.09 ± 0.02
Caryophyllene oxyde	1571	1980	1.24 ± 0.23	1.57 ± 0.25	4.07 ± 1.04	1.03 ± 0.1
1,10-di-epi-Cubenol	1605	2056	0.19 ± 0.05	0.19 ± 0.06	0.19 ± 0.1	0.47 ± 0.24
α-Cadinol	1641	2227	0.29 ± 0.1	0.74 ± 0.14	0.68 ± 0.4	1.03 ± 0.24
Total identified [%]			99.74	99814	99.62	99.77
Monoterpenes hydrocarbons			4.68 ± 0.74	7.86 ± 0.95	10.75 ±1.54	12.11 ± 0.98
Oxygenated monoterpenes			84.46 ± 4.22	76.50 ± 2.2	80.73± 3.34	81.97 ± 4.03
Sesquiterpene hydrocarbons			5.16 ± 1.2	8.05 ± 0.87	2.13 ± 0.74	2.14 ± 0.23
Oxygenated sesquiterpene			5.44 ± 1.15	7.400 ± 0.91	6.00 ± 1.22	3.54 ± 0.6

3.2. Antibacterial activity

In vitro antibacterial activity of *O.grosii and O.elongatum* essential oils (all sites) were assessed against 18 bacteria strains. The susceptibility or resistance profile of the strains has been studied against commercial antibiotics (Table3). The results showed in table 4 and table 5 was based on the inhibition zones of essential oils including the

diameter (6 mm) of the paper disk, The minimum inhibitory concentration (MIC;mg/mL) and minimum bactericidal concentration (MBC;mg/mL).

Essential oils from *O.grosii* were strongly active against all bacteria strains for the first and second altitude ranged between (16±0,6mm- 58±0,5mm) and (22±0,5mm- 52±0,8mm) respectively. The higher diameter was observed against *Enterobacter cloacae* (Bu5124) 58±0.5mm for the first altitude of *O.grosii* essential oil whereas, the second one was higher against *A.baumannii* (ASP195) 52±0.8mm. *S.marcescens* (H2451) was the most resistant bacteria (16±0.6) for the first altitude and most sensitive for the second (22±0,5mm), this value was also scored for *K.pneumonia* (P596) in the same site and it has the lower inhibition zone (table 4).

Yet for the second species (table 5), the inhibition zone of *O.elongatum* essential oils ranged between(10±0.2mm-52±1mm) and (18±0.5mm-62±0.7mm) respectively for the first and second altitudes. The *O.elongatum* essential oil was stranger against *E.faecalis* (P647) 54±0.5mm for the first altitude, while *A.baumannii* (HC2469) was the most sensitive bacteria for the second one 62±0.7mm. The lower value was observed against *k.pneumoniae* (Bu 4843) 10±0.2mm close to the inhibition zone 14±0.1mm observed for *P.aeruginosa* (P612) and *S.marcescens* (H2451) in the first altitude, however *S.marcescens* (ASP222) was the most resistant in the second altitude 18±0.5mm.

Table 3: The sensibility of bacteria isolated from clinical infection to commercial antibiotics.

	A. baumannii						*P. aeruginosa*		*E. cloacae*	*E.coli*		*K.pneumoniae*		*S.marcescens*	*E. faecalis*		*S. eureus*
Antibiotic	ASP 195	HC2243	p 582	HC2469	P 658	HC 2444	P 612	HC 2418	Bu 5124	HC2420	P561	P596	Bu 4843	H2451	ASP 222	P647	P631	H2463
Amoxicilline	R	R	R	R	R	R	S	R	R	R	S	R	R	S	R	S	R	R
Ticarcilline	R	R	R	R	R	R	S	S	R	R	S	R	R	S	S	S	S	S
Pipéracilline	R	R	R	R	R	R	S	S	R	S	S	R	R	S	S	S	S	S
Amoxicilline-clavulanate	R	R	R	R	R	R	S	R	R	R	S	R	S	S	R	S	S	S
Céfalotine	R	R	R	R	R	R	S	R	R	S	S	R	R	S	R	S	S	S
Céfoxitine	R	R	R	R	R	R	S	R	R	S	S	S	S	S	S	S	S	S
Céfixime	R	S	R	R	R	R	S	S	R	S	S	R	R	S	S	S	S	S
Céftazidime	R	R	R	R	R	R	S	S	R	S	S	R	R	S	S	S	S	S
Céfotaxime	R	R	R	R	R	R	S	S	R	S	S	R	R	S	S	S	S	S
Imipénéme	R	S	S	R	R	R	S	S	I	S	S	S	S	S	S	S	S	S
Ertapenem	R	R	R	R	R	R	S	R	I	S	S	S	S	S	S	S	S	S
Méropénéme	R	S	S	R	R	R	S	S	R	S	S	S	S	S	S	S	S	S
Piperacilline-Tazobactam	R	R	R	R	R	R	S	S	R	R	S	R	R	S	S	S	S	S
Aztréonam	R	R	R	R	R	R	S	S	R	S	S	R	R	S	S	S	S	S
Ciprofloxacine	R	R	R	R	R	R	S	S	I	S	S	S	R	S	S	S	S	S
Tobramycine	R	R	R	R	R	R	S	S	R	S	S	R	R	S	R	S	S	S
Gentamicine	R	R	R	R	R	R	R	S	R	S	S	R	R	S	S	S	S	S
Amikacine	R	S	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S
Triméthoprime-sulf	R	R	R	R	R	R	S	S	R	R	S	R	R	S	S	S	S	S
Céfépime	R	R	R	R	R	R	S	S	R	S	S	R	R	S	S	S	S	S
Tigécycline	R	R	R	R	R	R	S	S	S	S	S	R	R	S	S	S	S	S
Colistine	S	R	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S
Penicelline	R	R	R	R	R	R	S	S	R	S	S	R	R	S	S	S	R	R
Tecoplanine	R	R	R	R	R	R	S	S	R	S	S	R	R	S	S	S	S	S
Tétrocycline	R	R	R	R	R	R	S	S	R	S	S	R	R	S	S	S	S	S
Moxyfloxacine	R	R	R	R	R	R	S	S	R	S	S	S	R	S	S	S	S	S

Table 4: Inhibition zones, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of *O.grosii* essential oils for both altitudes.

		O.grosii (altitude1 : 1331m)			*O.grosii* (altitude2 : 797m)		
Bacteria	Code	Inhibition zone (mm)	MIC mg/mL	MBC mg/mL	Inhibition zone (mm)	MIC mg/mL	MBC mg/mL
A.baumannii	ASP 195	52±0,6	5	5	52±0,8	5	5
	HC2243	30±0,5	2.5	5	42±0,7	2.5	5
	P582	52±0,5	2.5	2.5	42±0,5	2.5	2.5
	HC2469	36±0,5	5	5	50±0,9	5	5
	P658	50±1	2.5	2.5	42±0,3	2.5	2.5
	HC 2444	50±0,6	5	5	38±0,5	5	5
P.aeruginosa	P612	20±0,2	10	10	24±0,2	10	10
	HC2418	46±0,5	2.5	2.5	42±0,3	2.5	2.5
E. cloacae	Bu 5124	58±0,5	5	5	38±0,6	5	5
E.coli	HC2420	30±0,3	5	10	40±0,5	5	10
	P561	24±0,2	2.5	5	28±0,3	2.5	5
k.pneumoniae	P596	24±0,6	5	5	22±0,3	5	5
	Bu 4843	28±0,5	5	10	24±0,3	5	10
S.marcescens	H2451	16±0,6	5	10	22±0,5	5	10
	ASP222	20±0,3	5	5	34±0,5	5	5
E. faecalis	P647	36±0,2	5	10	40±0,3	5	10
S. eureus	P631	32±0,2	1.3	1.3	34±0,3	5	5
	H2463	30±0,2	1.3	1.3	40±0,3	5	5

Table 5: Inhibition zones, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of *O.elongatum* essential oils for both altitudes

		O.elongatum (altitude1 : 923m)			*O.elongatum* (altitude2 : 1261m)		
Bacteria	Code	Inhibition zone (mm)	MIC mg/mL	MBC mg/mL	Inhibition zone (mm)	MIC mg/mL	MBC mg/mL
A.baumannii	ASP 195	52±1	5	5	5,8±0,9	5	5
	HC2243	34±0,6	2.5	2.5	4±0,5	2.5	2.5
	P582	46±0,5	2.5	2.5	5,6±0,2	2.5	2.5
	HC2469	42±0,5	5	5	6,2±0,7	5	5
	P658	34±0,5	2.5	2.5	5,6±0,5	2.5	2.5
	HC 2444	40±0,5	2.5	5	5±0,9	2.5	5
P.aeruginosa	P612	14±0,1	10	10	2,2±0,2	10	10
	HC2418	34±0,3	2.5	2.5	2,6±0,2	2.5	2.5
E. cloacae	Bu 5124	50±0,5	5	5	5,4±0,2	5	5
E.coli	HC2420	30±0,6	5	10	4±0,2	2.5	5
	P561	24±0,2	2.5	2.5	3,4±0,3	2.5	2.5
k.pneumoniae	P596	38±0,5	2.5	5	4,8±0,3	2.5	5
	Bu 4843	10±0,2	2.5	10	2,2±0,6	2.5	10
S.marcescens	H2451	14±0,2	5	5	2,6±0,5	5	5
	ASP222	26±0,6	2.5	5	1,8±0,5	2.5	10
E. faecalis	P647	54±0,5	5	5	4,8±0,5	5	5
S. eureus	P631	50±0,2	1.3	1.3	5±0,9	2.5	5
	H2463	50±0,9	1.3	1.3	4,2±0,3	5	5

The maximum values observed for both species *O.grosii* and *O.elongatum* essential oils were against

A.baumannii(52±0.8mm) and (62±0.7mm) in the second altitude. *P.aeruginosa* was most sensitive for *O.grosii* ranged between 20±0.2mm and 46±0.5mm comparing to *O.elongatum* ranged between 14±0.1mm and 34±0.3mm. The results observed against *E.coli* was equivalent for both species. The *O.elongatum* essential oils were stranger against *S.eureus* (ranged between 42±0.3mm and 50±0.2mm) comparing to *O.grosii* essential oils (ranged between 30±0.2mm and 40±0.3mm). It is very clear from these values that the activity differs according to the species, altitude and the bacteria, which can be explained by the impact of chemical composition differences.

Several works done on the antimicrobial mechanism of Carvacrol action (important proportion in both species), show that Carvacrol presents an antimicrobial activity on the biological membrane of bacteria exerts its action by rapidly depleting intracellular Adenosine-5'-triphosphate (ATP) [40]. Another study reported by Husnu Can Baser [41] declared that Carvacrol and Thymol had high antibacterial activity against Gram- negative bacteria like *Escherichia coli* and *Pseudomonas aeruginosa*, Gram-positive bacteria as *Staphylococcus aureus*, and *Enterococcus faecalis*. Also, Burt [42] reports that Carvacrol and Thymol inhibit the growth of *Escherichia coli* in liquid food. Ngome [43] declared that Linalol (appreciable proportion in both species) and Thymol had also a high bactericidal activity against several bacteria. Other studies report that Camphor has significant antibacterial activities [44, 45]. These compounds can be responsible for the antibacterial activity of the tested essential oils. Serval studies have also shown that other compounds (presented in small quantities in our samples) are characterized by their antibacterial properties such as Fenchone and 1,8- Cineole [46, 47] which demonstrate the potential of our samples as antibacterial agents.

In addition, the antibacterial activity was obtained via the determination of bacteriostatic and bactericidal concentrations. The minimum inhibitory concentration (MIC;mg/mL) and minimum bactericidal concentration (MBC;mg/mL) of the tested species essential oils against 18 strains are shown in table 5 and 6. *O.grosii* and *O.elongatum* showed the highest sensitivity against *Staphylocoque eureus* (P631;H2463) (MIC=MBC=1.3mg/mL;0.16%) for the first altitude while in the second, essential oils was stranger against P.aeruginosa (HC2418) (MIC=MBC=2.5mg/mL;0.31%). The (MIC=MBC=2.5mg/mL;0.31%) was observed also against *A.baumannii* (P582) for all tested essential oils.

According to our best knowledge, there is no work reported on the antibacterial activity of *O.grosii*. El Harsal [8] reported that *O.elongatum* had antibacterial activity against *E.coli* and *S.aureus* MIC=0.5% and MIC=0.12%, respectively, which are partially in accordance with our results. However, our results are promising in terms of efficacy if compared to previous works reported MIC proportion [48]: *S.aureus* MIC=0.125% and *P.aeruginosa* MIC=2% .

4. **Conclusion**

The fight against bacterial infections and pathogenic bacteria, especially those that resistance to antibiotics, is the main objective for most scientists and researchers. *Origanum grosii*, and *Origanum elongatum* essential oils have shown antibacterial activity against a lot of resistant bacteria strains. The results obtained in this study indicate that the chemical composition of *O.grosii* and *O.elongatum* essential oils for all altitudes vary depending on several factors (altitude, climatic, edaphic condition…) which impact on its antibacterial activity. The revealed major compounds are known for their strong biological activities, especially Carvacrol and Thymol, taking into account those are present in small quantities which are also characterized by their antibacterial activity. *O.grosii* and *O.elongatum* essential oils may be suggested as a new source of antibacterial agents after testing its toxicity on humans. Those compounds could be used in the pharmaceutical and food industries as natural products. In addition, the synergic effect of the essential oil components can be also responsible for this activity.

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